Clinical and epidemiological characteristics of acute respiratory virus infections in Vietnamese children

D. N. TRAN[1,2,3], Q. D. TRINH[3], N. T. K. PHAM[3], M. P. VU[2], M. T. HA[4],
T. Q. N. NGUYEN[1,4], S. OKITSU[1,3], S. HAYAKAWA[3], M. MIZUGUCHI[1]
AND H. USHIJIMA[1,3]*

[1] *Department of Developmental Medical Sciences, School of International Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*
[2] *Department of Pediatrics, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam*
[3] *Division of Microbiology, Department of Pathology and Microbiology, Nihon University School of Medicine, Tokyo, Japan*
[4] *Children's Hospital 2, Ho Chi Minh City, Vietnam*

SUMMARY

Information about viral acute respiratory infections (ARIs) is essential for prevention, diagnosis and treatment, but it is limited in tropical developing countries. This study described the clinical and epidemiological characteristics of ARIs in children hospitalized in Vietnam. Nasopharyngeal samples were collected from children with ARIs at Ho Chi Minh City Children's Hospital 2 between April 2010 and May 2011 in order to detect respiratory viruses by polymerase chain reaction. Viruses were found in 64% of 1082 patients, with 12% being co-infections. The leading detected viruses were human rhinovirus (HRV; 30%), respiratory syncytial virus (RSV; 23·8%), and human bocavirus (HBoV; 7·2%). HRV was detected all year round, while RSV epidemics occurred mainly in the rainy season. Influenza A (FluA) was found in both seasons. The other viruses were predominant in the dry season. HRV was identified in children of all age groups. RSV, parainfluenza virus (PIV) 1, PIV3 and HBoV, and FluA were detected predominantly in children aged <6 months, 6–12 months, 12–24 months, and >24 months, respectively. Significant associations were found between PIV1 with croup ($P < 0.005$) and RSV with bronchiolitis ($P < 0.005$). HBoV and HRV were associated with hypoxia ($P < 0.05$) and RSV with retraction ($P < 0.05$). HRV, RSV, and HBoV were detected most frequently and they may increase the severity of ARIs in children.

Key words: Acute respiratory infection, children, epidemiology, Vietnam.

INTRODUCTION

Acute respiratory infections (ARIs) are a leading cause of morbidity and mortality in infants and young children worldwide. About 1 million children aged <5 years died from pneumonia in 2013, accounting for 15% of all deaths in this age group, with most of them in developing countries [1]. In addition, ARIs substantially burden patients and their families, with direct medical expenses and indirect costs from missed workdays or absence from school and day care [2].

Several severe outbreaks such as SARS-coronavirus, avian and H1N1 2009 pandemic influenza occurred

* Author for correspondence: H. Ushijima, M.D., Ph.D., Division of Microbiology, Department of Pathology and Microbiology, Nihon University, School of Medicine, 30-1 Oyaguchi-Kamicho, Itabashi-ku, Tokyo 173-8610, Japan.
(Email: ushijima-hiroshi@jcom.home.ne.jp)

recently in Southeast Asia, which makes ARIs an increasingly serious public health problem. Viruses are the most common cause of ARIs and a major reason for hospitalization in young children [3]. However, due to limited resources and facilities, the role of individual respiratory virus in these settings is not well documented. Better understanding of clinical and epidemiological characteristics of ARIs is essential for predicting epidemics, estimating aetiological agents, and establishing effective prevention and treatment measures. Therefore, this study was conducted to identify common respiratory viruses in hospitalized Vietnamese children with ARIs and describe their epidemiological and clinical features, as well as to investigate their effect on disease severity of ARIs.

MATERIALS AND METHODS

Study site and patients

The study was conducted from 1 April 2010 to 31 May 2011 at the Respiratory Ward, Children's Hospital 2, Ho Chi Minh City, Vietnam. Children's Hospital 2 is a 1400-bed tertiary referral and university-affiliated hospital, receiving paediatric patients from most parts of the city as well as other provinces in south and southern central Vietnam. This area has a tropical climate with two distinct seasons: rainy season (May–October) and dry season (November-April). The temperature does not change much during the year, and varied between 24 °C and 32 °C during the time of this study.

Children eligible for inclusion in this study were aged <15 years, and admitted to hospital <7 days after ARI onset. An ARI case was defined as a child presenting with cough and/or difficulty in breathing [4]. Patients who had underlying chronic diseases (e.g. cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease, and immunodeficiency), or were discharged from hospital within the previous 7 days, as well as those with co-existing acute systemic illnesses (e.g. sepsis), or proven or suspected non-infectious respiratory symptoms (e.g. asthma), were excluded from the study. Patients with previous respiratory infection, within 3 weeks of current hospitalization, were excluded as well in order to avoid prolonged viral shedding.

Clinical data

After signed consent was obtained from the children's parents/guardians, demographic and clinical data were recorded on a standardized questionnaire. Demographic parameters included gender, age at admission, birth weight, and gestational age. Clinical observations at admission included vital signs, symptoms and signs contributory to the diagnosis of ARI, i.e. presence of wheezing and chest retraction, and transcutaneous oxygen saturation, as measured by a portable pulse oximeter (Siemens Micro O2, Siemens Medical Systems Inc., USA) using a paediatric sensor (Nellcor, USA). The laboratory workup included complete blood counts (CBC) and a chest X-ray (CXR). In cases where more than one CBC had been affected, only the first count was recorded. Determination of bacteria was not routinely carried out at admission. Only cases with systemic sepsis or prolonged pneumonia were subjected to blood and sputum bacterial culture. The treatment course (oxygen support, and antibiotic, steroid and bronchodilator therapy) and outcome (duration of hospitalization and complications) were noted.

The diagnosis was made on the basis of clinical findings and CXR. ARI with the presence of an infiltrate on the CXR was categorized as pneumonia [5]. Bronchiolitis was defined as an ARI patient aged <2 years presenting with wheezing and hyperaeration, atelectasis, or peribronchial thickening on the CXR [6]. Croup was characterized by hoarseness, cough, and stridor [7]. Upper respiratory tract infection (URTI) was defined as ARI with no abnormalities on the CXR.

Clinical samples and virus detection

Nasopharyngeal (NP) flocked swabs (MicroRheologics, Italy) were obtained from all participants by trained personnel within 24 h post-admission. The specimens were stored immediately at –20 °C at the laboratory until further analysis. Viral genomes were extracted directly from the respiratory specimens by using the QIAamp Viral RNA mini-kit (Qiagen, Germany), according to the manufacturer's instructions, and stored at −80 °C. Four multiplex (semi-)nested polymerase chain reaction (PCR) assays were used to detect 13 respiratory viruses, including respiratory syncytial virus (RSV), influenza virus (Flu) A and B, human metapneumovirus (hMPV), parainfluenza virus (PIV) types 1–4, human rhinovirus (HRV), human coronavirus (HCoV) OC43 and 229E, adenovirus (AdV) and human bocavirus (HBoV), as described previously [8].

Statistical analysis

Demographic and clinical characteristics of virus-positive patients were compared to virus-negative

patients. Values were given as percentages for categorical variables, and as median with interquartile range (IQR) for continuous variables. Bivariate associations were assessed using the χ^2 test or Fisher's exact test for categorical variables, and Mann–Whitney U test for continuous variables. A two-sided value of $P < 0.05$ was considered statistically significant.

Multivariate logistic regression analyses were performed to determine the association of a specific type of detected virus (HRV, RSV, HBoV, PIV1, PIV3, FluA) with severe symptoms and diagnostic classification. The outcomes were tachypnoea, retraction and $SpO_2 \leqslant 92\%$ for severe symptoms, and URTIs, croup, bronchiolitis and pneumonia for diagnostic classification. Univariate models were performed initially for introducing independent variables such as age, gender, prematurity, malnutrition, co-infection with other viruses and type of detected virus. Subsequently, these variables (known as potential confounders) were introduced in a multivariate model with a step-wise approach, to eliminate the possibility of mutual confounding and interaction. Results are presented as odds ratios (ORs) with 95% confidence intervals (95% CIs). ORs and 95% CIs that did not contain 1 were considered significant.

All analyses were conducted using SPSS software version 16·0 (SPSS Inc., USA).

Ethical standards

The study was approved by the Scientific and Ethical Committee of Children's Hospital 2, Ho Chi Minh City, Vietnam (no. 25A/QD-ND2) and Nihon University School of Medicine, Tokyo, Japan (no. 25-15-0). Written informed consent was obtained from the parents or legal guardians of all the children enrolled in this study.

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

RESULTS

Characteristics of the study population

This study enrolled 1082 patients with ARIs during a 14-month period. The demographic and clinical characteristics of this study population are given in Table 1. Overall, the median age of the participants was 9 months (range 0–161 months), with 86% aged <2 years. Males were affected more commonly than females (male:female ratio 1·8:1). Most patients had fever, cough, and runny nose on admission to hospital. On examination, the percentage of patients with fast breathing, wheezing and chest retraction was 44·5%, 58·9% and 55·3%, respectively. Lower respiratory tract infections (LRTIs) were diagnosed in 78·4% of cases. Seventy-six percent of patients received antibiotic treatment during hospitalization. No patients required mechanical ventilation or had complications. All patients fully recovered and were discharged.

Virus detection and seasonal patterns

One or more respiratory viruses were detected in 64·6% (699/1082) of the patients (Table 2). Viruses could be found in all months of the study period, and the monthly detection rates ranged from 47·4% to 90·2%. ARI activity peaked in August, September and October and detection rates were also high during these months (71·2%, 80·2% and 90·2%, respectively). HRV was the most common virus identified in the hospitalized patients, with an overall detection rate of 30%. RSV was the second most frequently found (23·8%), followed by HBoV, PIV3, PIV1, and FluA. Other viruses such as hMPV, AdV, FluB and HCoV were detected in small proportions. Neither PIV2 nor PIV4 were found in this study. Mixed infection of more than one respiratory virus was found in 129 (12%) patients, in which, co-infection between HRV and RSV was the most frequent (48 cases), followed by combinations of HRV and HBoV (16 cases). It was noted that 66·7% (52/78) of HBoV and 57·14% (4/7) of HCoV OC43 were mixed infections with other viruses. Six patients had triple infection.

Regarding seasonal patterns, HRV was detected all year round with no distinct seasonality (Fig. 1). An RSV epidemic occurred during the rainy season from May to October with a peak in October 2010. By contrast, PIV1, PIV3 and HBoV were predominant during the dry season. Interestingly, FluA was found in both rainy and dry seasons. The number of remaining respiratory viruses was insufficient for detecting seasonal patterns.

Clinical findings associated with type of respiratory viruses

To differentiate between virus-positive and virus-negative groups, clinical and demographic data were

Table 1. *Demographic and clinical data of the study population and virus-positive and virus-negative groups*

Characteristics (%)	Total (N = 1082)	Virus pos. (N = 699)	Virus neg. (N = 383)	P value*	OR (95% CI)
Male	64·7	66·7	61·1	0·073	
Age, months, median (IQR)	9 (4–18)	9 (4–17)	10 (4–19)	0·250	
Age group, months				**0·046†**	
<6	33·0	32·3	34·2	0·543	
6−<12	23·9	26·6	19·1	**0·006†**	1·54 (1·14–2·10)
12−<24	27·4	26·0	29·8	0·200	
⩾24	15·7	15·0	17·0	0·432	
Prematurity	9·2	10·0	7·8	0·273	
Malnutrition	9·5	8·4	11·5	0·105	
Fever	67·2	66·7	68·1	0·636	
Cough	90·9	92·3	88·3	**0·036†**	1·59 (1·05–2·41)
Runny nose	73·4	78·3	64·5	**<0·001†**	1·98 (1·50–2·61)
SpO$_2$ ⩽92%	8·5	8·4	8·6	0·910	
Tachypnoea	44·5	42·5	48·3	0·073	
Chest retraction	55·3	58·9	48·3	**0·001†**	1·54 (1·20–1·97)
Wheezing	58·9	60·7	55·6	0·121	
Rales	66·0	68·0	62·4	0·070	
WBC (×10^3/mm^3), median (IQR)	11·6 (9·392–15)	11·4 (9·39–14·6)	12·2 (9·40–15·5)	**0·037‡**	
Abnormal CXR	77·6	81·3	70·7	**<0·001†**	1·80 (1·35–2·42)
Diagnosis				**0·007†**	
URTIs	21·6	21·6	21·7	1·000	
Croup	6·1	4·7	8·6	**0·016†**	0·53 (0·32–0·87)
Bronchiolitis	33·2	36·1	27·9	**0·007†**	1·45 (1·11–1·91)
Pneumonia	39·1	37·6	41·8	0·193	
Oxygen	8·0	8·2	7·8	0·907	
Antibiotics	76·0	76·0	76·0	1·000	
Steroids	15·7	16·3	14·6	0·486	
Bronchodilator	51·8	56·9	42·6	**<0·001†**	1·79 (1·39–2·30)
Hospitalization duration, days, median (IQR)	6 (4–8)	6 (4–8)	5 (3–8)	0·203	

Pos, Positive; neg, negative; OR, odds ratio; CI, confidence interval; IQR, interquartile range; WBC, white blood cell; URTI, upper respiratory tract infection.

All results are expressed in percentages except where stated otherwise.

* Comparison between virus positive and negative groups.

† χ^2 test.

‡ Mann–Whitney U test.

compared (Table 1). With regard to age, infants aged from 6 to 12 months had almost twice as much risk of viral infections than other age groups (OR 1·54, 95% CI 1·14-2·10). Virus-positive patients had significantly higher rates of cough (92·3 *vs.* 88·3%, P = 0·036), runny nose (78·3 *vs.* 64·5%, P < 0·001), and chest retraction (58·9 *vs.* 48·3%, P = 0·001) than virus-negative patients. However, the latter group was likely to have a higher number of white blood cells (WBCs) (12 200 *vs.* 11 400, P = 0·037). The virus-positive group had more abnormal radiographs than the virus-negative group (81·3 *vs.* 70·7%, P < 0·001). Regarding diagnosis, virus-positive patients were more likely to have bronchiolitis (36·1 *vs.* 27·9%, P = 0·007), but less likely to have croup

(4·7 *vs.* 8·6%, P = 0·016) than virus-negative patients. As a result, the former group received bronchiodilators more often than the latter (56·9 *vs.* 42·6%, P < 0·001). There were no significant differences in other characteristics, as shown in Table 1. Clinical characteristics of patients with co-infections were not significantly different to those with a single infection (data not shown).

Each type of respiratory virus that associated with specific demographic and clinical features was also found. The age distribution of patients was specific for each type of virus (Fig. 2). In particular, HRV was detected in children of all age groups, whereas RSV was found usually in children aged <6 months, while FluA was found mostly in children aged

Table 2. *Number of single and multiple viruses detected in 1082 patients with ARIs*

Virus	HRV	RSV	HBoV	PIV3	PIV1	Flu A	hMPV	AdV	Flu B	HCoV 229E	HCoV OC43
HRV	234	48	16	11	3	3	1	2	1	0	0
RSV	48	184	15	1	1	1	0	0	0	0	3
HBoV	16	15	26	6	2	4	2	0	2	0	0
PIV3	11	1	6	38	0	0	0	0	0	0	0
PIV1	3	1	2	0	29	0	1	0	0	0	0
FluA	3	1	4	0	0	26	0	0	0	0	0
hMPV	1	0	2	0	1	0	9	0	0	0	0
AdV	2	0	0	0	0	0	0	9	0	0	0
FluB	1	0	2	0	0	0	0	0	4	0	0
HCoV 229E	0	0	0	0	0	0	0	0	0	7	0
HCoV OC43	0	3	0	0	0	0	0	0	0	0	4
HRV + RSV + HBoV	3	3	3	0	0	0	0	0	0	0	0
HRV + RSV + AdV	1	1	0	0	0	0	0	1	0	0	0
HRV + Flu A + HBoV	1	0	1	0	0	1	0	0	0	0	0
HRV + PIV3 + HBoV	1	0	1	1	0	0	0	0	0	0	0
Total*	325 (30%)	257 (23·8%)	78 (7·2%)	57 (5·3%)	36 (3·3%)	35 (3·2%)	13 (1·2%)	12 (1·1%)	7 (0·6%)	7 (0·6%)	7 (0·6%)

HRV, Human rhinovirus; RSV, respiratory syncytial virus; HBoV, human bocavirus; PIV, parainfluenza virus; Flu, influenza virus; hMPV, human metapneumovirus; AdV, adenovirus; HCoV, human coronavirus.

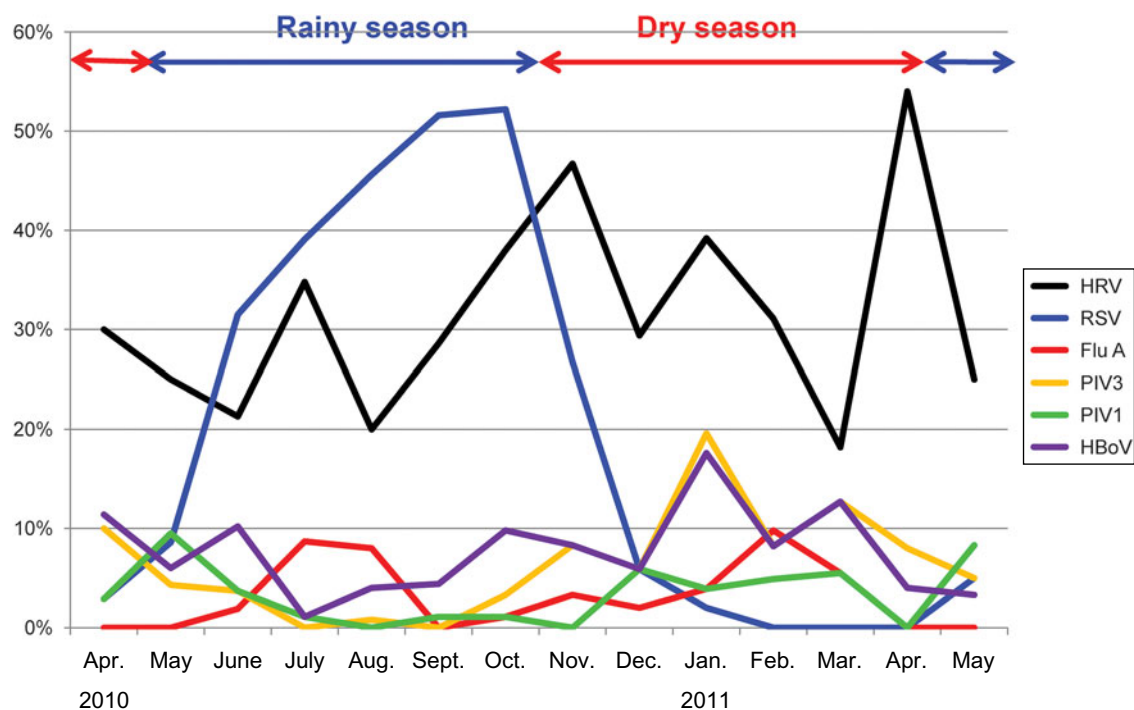* Percentages are calculated based on the positive number of each virus.

Fig. 1. Monthly distribution of leading viral agents in hospitalized children with acute respiratory infections from April 2010 to May 2011. HRV, Human rhinovirus; RSV, respiratory syncytial virus; FluA, influenza A virus; PIV, parainfluenza virus; HBoV, human bocavirus.
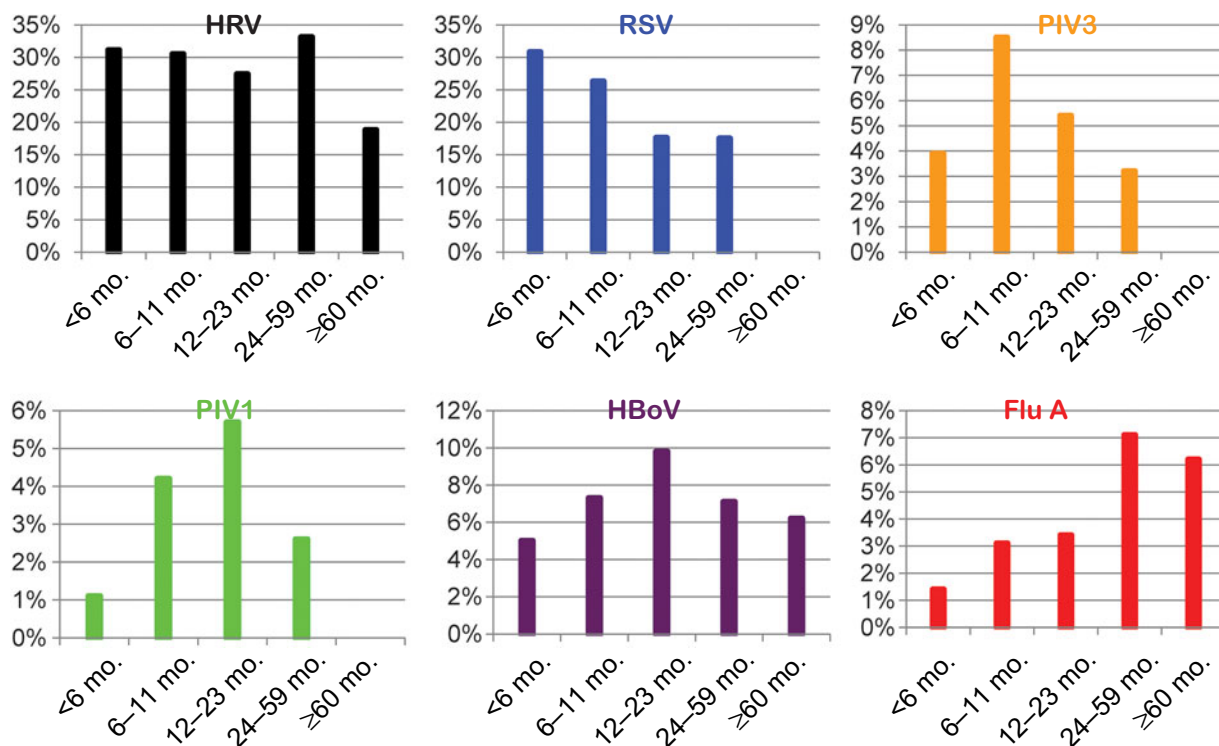
Fig. 2. Age distribution of leading viral agents. HRV, human rhinovirus; RSV, respiratory syncytial virus; PIV, parainfluenza virus; HBoV, human bocavirus; FluA, influenza A virus.

>2 years. PIV3 developed ARIs frequently in children aged 6–11 months, which was in contrast to PIV1 and HBoV that were predominant in the second year of life.

The effect of respiratory viruses on the risk of having severe clinical signs is shown in Table 3. By univariate analysis, HRV and RSV were associated with retraction, while HRV and HBoV were associated with hypoxia. By multivariate analysis adjusting for age, gender, prematurity, malnutrition and other viral co-infections, HRV and RSV were independent risk factors for retraction, whereas HRV and HBoV were independent risk factors for hypoxia. Regarding diagnosis, RSV was associated with bronchiolitis, and PIV1 with croup in both univariate and multivariate analysis (Table 4). HBoV was associated with pneumonia in the univariate analysis, but this association became borderline in multivariate analysis (adjusted OR 1·59, 95% CI 0·99–2·55). It was noteworthy that HRV or RSV and P1V1 detection was less likely to associate with croup and bronchiolitis, respectively. Other viruses such as FluB, hMPV, HCoV, and AdV were not included in this analysis, due to the small number of cases in each category.

DISCUSSION

Virus detection and seasonal patterns

In this study we describe a wide variety of viruses associating with ARIs in southern Vietnamese children. The identification of 11 different respiratory viruses in two-thirds of hospitalized patients with ARIs was concordant with previous studies in Vietnam [8, 9]. Altogether, these studies confirmed the burden of viral ARIs in this area. Viral detection rates peaked during months when a high number of ARI patients were hospitalized (August–October). Simultaneously, RSV detection also peaked during this time. Therefore, it could be inferred that ARI epidemics were driven to some extent by viral infections, especially RSV infections.

HRV was the most common virus detected, followed by RSV and HBoV. These three viruses accounted for 83·1% of virus-positive patients, thus indicating their importance in hospitalization of children with ARIs in southern Vietnam. This result is consistent with previous studies in Europe [10–13] and Southeast Asia [8, 14]. FluA, one of the major causes of ARIs, was detected in a small proportion of patients. According to the triage process of the

Table 3. *Association between a specific respiratory virus and severe clinical signs*

Virus*	Yes	No	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Tachypnoea, *n* (%)				
HRV	133 (40·9)	192 (59·1)	0·81 (0·62–1·05)	
RSV	107 (41·6)	150 (58·4)	0·86 (0·65–1·14)	
HBoV	36 (46·2)	42 (53·8)	1·07 (0·68–1·70)	
PIV3	19 (33·3)	38 (66·7)	0·61 (0·35–1·07)	
PIV1	19 (52·8)	17 (47·2)	1·41 (0·72–2·74)	
FluA	21 (60·0)	14 (40·0)	1·91 (0·96–3·79)	
Retraction, *n* (%)				
HRV	198 (60·9)	127 (39·1)	1·40 (1·07–1·82)	1·49 (1·13–1·96)
RSV	176 (68·5)	81 (31·5)	2·09 (1·55–2·81)	1·96 (1·44–2·68)
HBoV	43 (55·1)	35 (44·9)	1·00 (0·63–1·59)	
PIV3	26 (45·6)	31 (54·4)	0·67 (0·39–1·14)	
PIV1	13 (36·1)	23 (63·9)	0·45 (0·22–0·89)	0·55 (0·27–1·13)
FluA	17 (48·6)	18 (51·4)	0·76 (0·39–1·49)	
SpO$_2$ ⩽92%, *n* (%)				
HRV	40 (12·3)	285 (87·7)	1·90 (1·23–2·94)	1·78 (1·14–2·79)
RSV	15 (5·8)	242 (94·2)	0·60 (0·34–1·07)	
HBoV	13 (16·7)	65 (83·3)	2·34 (1·24–4·43)	2·32 (1·22–4·41)
PIV3	2 (3·5)	55 (96·5)	0·38 (0·09–1·57)	
PIV1	1 (2·8)	35 (97·2)	0·30 (0·04–2·21)	
FluA	2 (5·7)	33 (94·3)	0·64 (0·15–2·73)	

HRV, Human rhinovirus; RSV, respiratory syncytial virus; HBoV, human bocavirus; PIV, parainfluenza virus; FluA, influenza A virus; OR, odds ratio; CI, confidence interval.
OR was adjusted for age, gender, prematurity, malnutrition, and co-infection with other viruses.
* Reference category comprised patients testing negative for the virus of interest.

hospital, patients with suspected influenza infections are isolated and treated at the Infectious Diseases Ward. Since only samples from the Respiratory Ward were collected, the number of influenza cases in this study may be an underrepresentation.

Moreover, the study period of only 14 months was too short to conclude seasonal patterns of different respiratory viruses. Nevertheless, some interesting points were found. The RSV epidemic peaked in the rainy season, which is different from temperate countries, where RSV infections usually occur in winter [15]. FluA in Vietnam was found in both rainy and dry seasons, which also was different to that in temperate countries. PIV infections are prevalent during autumn or spring in many temperate countries [3], and did not show significant annual seasonality in other tropical zones besides Vietnam [16], but their activity in this study occurred mainly during the dry season, which made up for a weak RSV season. Although the reasons for seasonality are quite unclear, determining seasonality of respiratory viruses is very important in guiding appropriate preventive strategies and clinical management.

Clinical findings associated with type of respiratory viruses

This study achieved statistical significance for the detection of viruses and their association with clinical severity. The virus-positive patients tended to have more severe symptoms. This result therefore confirms that the presence of viruses may have an effect on the clinical severity of ARIs. Although the virus-negative group had a higher WBC count, it was insufficient for concluding possible bacterial infection [17]. Children aged 6–12 months are at risk of virus infection because at this time maternal antibodies decrease markedly and the immune system is not mature enough to protect the infants from infection. Regarding diagnosis, potential viruses known to cause bronchiolitis such as RSV, HRV, and PIV3 accounted for a relatively large part in this study, and explained why this diagnosis was seen significantly more often in the virus-positive than virus-negative group. In this study, the relationship between overall co-infections and increasing disease severity could not be established. Recent studies have shown

Table 4. *Association between a specific respiratory virus and categories of diagnosis*

Virus*	Yes	No	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
URTIs, *n* (%)				
HRV	81 (24·9)	244 (75·1)	1·31 (0·96–1·78)	
RSV	41 (16·0)	216 (84·0)	0·62 (0·43–0·90)	0·73 (0·49–1·07)
HBoV	18 (23·1)	60 (76·9)	1·09 (0·63–1·89)	
PIV3	9 (15·8)	48 (84·2)	0·67 (0·32–1·38)	
PIV1	6 (16·7)	30 (83·3)	0·72 (0·30–1·75)	
FluA	8 (22·9)	27 (77·1)	1·08 (0·48–2·40)	
Bronchiolitis, *n* (%)				
HRV	118 (36·3)	207 (63·7)	1·22 (0·93–1·60)	
RSV	121 (47·1)	136 (52·9)	2·19 (1·65–2·93)	1·89 (1·37–2·61)
HBoV	18 (23·1)	60 (76·9)	0·58 (0·34–1·01)	
PIV3	22 (38·6)	35 (61·4)	1·28 (0·74–2·22)	
PIV1	4 (11·1)	32 (88·9)	0·24 (0·09–0·69)	0·33 (0·11–0·97)
FluA	5 (14·3)	30 (85·7)	0·33 (0·13–0·85)	0·50 (0·18–1·42)
Croup, *n* (%)				
HRV	9 (2·8)	316 (97·2)	0·35 (0·17–0·72)	0·31 (0·15–0·64)
RSV	2 (0·8)	255 (99·2)	0·09 (0·02–0·38)	0·08 (0·02–0·35)
HBoV	3 (3·8)	75 (96·2)	0·60 (0·18–1·95)	
PIV3	4 (7·0)	53 (93·0)	1·17 (0·41–3·34)	
PIV1	14 (38·9)	22 (61·1)	12·2 (5·89–25·14)	8·31 (3·88–17·81)
FluA	3 (8·6)	32 (91·4)	1·46 (0·44–4·91)	
Pneumonia, *n* (%)				
HRV	117 (36·0)	208 (64·0)	0·83 (0·63–1·09)	
RSV	93 (36·2)	164 (63·8)	0·85 (0·64–1·14)	
HBoV	39 (50·0)	39 (50·0)	1·62 (1·02–2·56)	1·59 (0·99–2·55)
PIV3	22 (38·6)	35 (61·4)	0·98 (0·57–1·69)	
PIV1	12 (33·3)	24 (66·7)	0·77 (0·38–1·56)	
FluA	19 (54·3)	16 (45·7)	1·89 (0·96–3·72)	

URTI, Upper respiratory tract infection; HRV, human rhinovirus; RSV, respiratory syncytial virus; HBoV, human bocavirus; PIV, parainfluenza virus; FluA, influenza A virus; OR, odds ratio; CI, confidence interval.
OR was adjusted for age, gender, prematurity, malnutrition, and co-infection with other viruses.
* Reference category comprised patients testing negative for the virus of interest.

that co-infections are not uncommon [18]; however, their effect on severity depends on the combination of different viruses [19].

Many viruses are responsible for ARIs and it is believed that the virus type may influence the clinical manifestations of infection. In this study, different age distribution of each type of virus was found. This information may be useful in clinical practice. As in previous literature, RSV is always associated with bronchiolitis. The fact that most RSV-positive cases were diagnosed as either bronchiolitis or pneumonia, and associated with chest retraction, demonstrates that RSV was by far the most important pathogen in children. This finding is comparable with results from other studies [13, 20, 21]. Croup was a clinical picture that associated uniquely with PIV1, as seen in another study [22].

Of note, HRV was the main reason for hospitalization with ARIs in this study and LRTIs were responsible for two-thirds of HRV-positive cases. In addition, HRV was associated with the development of severe symptoms such as hypoxia and retraction. A majority (75%) of HRV-positive patients had onset of symptoms within 5 days prior to hospital admission (data not shown), suggesting that HRV is a possible cause of these symptoms rather than bacterial superinfection in later presentation. Previous studies also showed that HRV has been detected increasingly and is associated with LRTIs in children [23, 24]. Furthermore, experiments have shown the ability of HRV to infect the lower respiratory tract [24–27]. Despite all these supporting data for the pathogenesis of HRV, caution must be exercised when interpreting its role, since it could be found in about 15% of

asymptomatic individuals, compared to <5% of most other respiratory viruses [28].

The newly discovered HBoV was detected in a substantial number of cases and represented the third most common virus in this study. Although significantly associated with hypoxia, HBoV was detected frequently in combination with other pathogens, and some studies showed it was often found in asymptomatic individuals [29]. On the other hand, several studies suggested that identification of single HBoV was more common in sick patients than in control subjects [29]. Therefore, the role of HBoV as a true pathogen remains to be determined.

Although clinical signs and symptoms of various respiratory viruses are overlapping, this work found different clinical characteristics associated with common respiratory viruses. Taken together with the differences in seasonal patterns and age distribution, it is possible to speculate the probability of diseases being caused by a specific virus. This may contribute to clinical benefits in developing country settings, where facilities for virus detection are not available.

Limitations of this study

This study has some limitations. Although a wide spectrum of diseases and severities were included, a large number of outpatients were not recruited. Therefore, the results of this study can be applied to hospitalized patients only. The detection of viruses in NP samples provides only indirect evidence of LRTI aetiology and, moreover, the study lacked a control group. Due to the sensitivity of PCR, it was possible to detect the remnant material of old pathogens together with current active pathogens. Using control samples to detect pathogens in asymptomatic children would help determine the likelihood of these viruses being true pathogens. Although the detection rate was high, viruses were still unidentified in nearly 35% of enrolled patients. It is possible that some children may have had infection with bacteria or other viruses not included in the assays of this study (e.g. enteroviruses, and other types of HCoV such as NL63 and HKU1), or with agents yet to be discovered. Information regarding bacterial infection was not available in this study, since obtaining appropriate sputum from the lower respiratory tract is not feasible in young children, and blood culture usually reveals low positive rates due to a high rate of antibiotic usage before hospitalization.

In conclusion, the results of this study confirmed the diversity of viruses associated with ARIs in children in southern Vietnam. HRV, RSV and HBoV were the leading viruses detected, and they may increase the severity of ARIs in children. Specific viruses were associated with specific clinical syndromes, specific seasonal patterns and age distribution. The findings from this study were useful for improving diagnosis, prevention and treatment activities, especially in resource-limited countries. More knowledge of viruses causing ARIs in children would help to decrease the disease burden, not only for Vietnam, but also for other countries. Similar surveillance on common viral agents should be continued in the future in order to understand the fluctuation of these viruses. A control group should be added so that the causative role of each virus can be explained more accurately.

DECLARATION OF INTEREST

None.

REFERENCES

1. **Liu L, et al.** Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 2015; **385**: 430–440.
2. **Fendrick AM, et al.** The economic burden of non-influenza-related viral respiratory tract infection in the United States. *Archives of Internal Medicine* 2003; **163**: 487–494.
3. **Mahony JB.** Detection of respiratory viruses by molecular methods. *Clinical Microbiology Reviews* 2008; **21**: 716–747.
4. **World Health Organization.** Pocket book of hospital care for children: guidelines for the management of common illnesses with limited resources. Geneva: World Health Organization, 2005.

5. **Bradley JS, et al.** The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clinical Infectious Diseases* 2011; **53**: e25–76.

6. **American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis.** Diagnosis and management of bronchiolitis. *Pediatrics* 2006; **118**: 1774–1793.

7. **Zoorob R, Sidani M, Murray J.** Croup: an overview. *American Family Physician* 2011; **83**: 1067–1073.

8. **Yoshida LM, et al.** Viral pathogens associated with acute respiratory infections in central vietnamese children. *Pediatric Infectious Disease Journal* 2010; **29**: 75–77.

9. **Do AH, et al.** Viral etiologies of acute respiratory infections among hospitalized Vietnamese children in Ho Chi Minh City, 2004–2008. *PLoS ONE* 2011; **6**: e18176.

10. **Calvo C, et al.** Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Acta Paediatrica* 2010; **99**: 883–887.

11. **Midulla F, et al.** Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. *Archives of Disease in Childhood* 2010; **95**: 35–41.

12. **Tregoning JS, Schwarze J.** Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clinical Microbiology Reviews* 2010; **23**: 74–98.

13. **Weigl JA, et al.** The descriptive epidemiology of severe lower respiratory tract infections in children in Kiel, Germany. *Klinische Pädiatrie* 2005; **217**: 259–267.

14. **Guerrier G, et al.** Acute viral lower respiratory tract infections in Cambodian children: clinical and epidemiologic characteristics. *Pediatric Infectious Disease Journal* 2013; **32**: e8–13.

15. **Tang JW, Loh TP.** Correlations between climate factors and incidence – a contributor to RSV seasonality. *Reviews in Medical Virology* 2014; **24**: 15–34.

16. **Chew FT, et al.** Seasonal trends of viral respiratory tract infections in the tropics. *Epidemiology and Infection* 1998; **121**: 121–128.

17. **Virkki R, et al.** Differentiation of bacterial and viral pneumonia in children. *Thorax* 2002; **57**: 438–441.

18. **Ruuskanen O, et al.** Viral pneumonia. *Lancet* 2011; **377**: 1264–1275.

19. **Goka EA, et al.** Single and multiple respiratory virus infections and severity of respiratory disease: a systematic review. *Paediatric Respiratory Reviews* 2014; **15**: 363–370.

20. **Berkley JA, et al.** Viral etiology of severe pneumonia among Kenyan infants and children. *Journal of the American Medical Association* 2010; **303**: 2051–2057.

21. **Weber MW, Mulholland EK, Greenwood BM.** Respiratory syncytial virus infection in tropical and developing countries. *Tropical medicine & international health* 1998; **3**: 268–280.

22. **Reed G, et al.** Epidemiology and clinical impact of para-influenza virus infections in otherwise healthy infants and young children <5 years old. *Journal of Infectious Diseases* 1997; **175**: 807–813.

23. **Hayden FG.** Rhinovirus and the lower respiratory tract. *Reviews in Medical Virology* 2004; **14**: 17–31.

24. **Papadopoulos NG, et al.** Rhinoviruses infect the lower airways. *Journal of Infectious Diseases* 2000; **181**: 1875–1884.

25. **Kaiser L, et al.** Chronic rhinoviral infection in lung transplant recipients. *American Journal of Respiratory and Critical Care Medicine* 2006; **174**: 1392–1399.

26. **Malmstrom K, et al.** Human rhinovirus in bronchial epithelium of infants with recurrent respiratory symptoms. *Journal of Allergy and Clinical Immunology* 2006; **118**: 591–596.

27. **Mosser AG, et al.** Quantitative and qualitative analysis of rhinovirus infection in bronchial tissues. *American Journal of Respiratory and Critical Care Medicine* 2005; **171**: 645–651.

28. **Jartti T, et al.** Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections. *Pediatric Infectious Disease Journal* 2008; **27**: 1103–1107.

29. **Jartti T, et al.** Human bocavirus – the first 5 years. *Reviews in Medical Virology* 2012; **22**: 46–64.